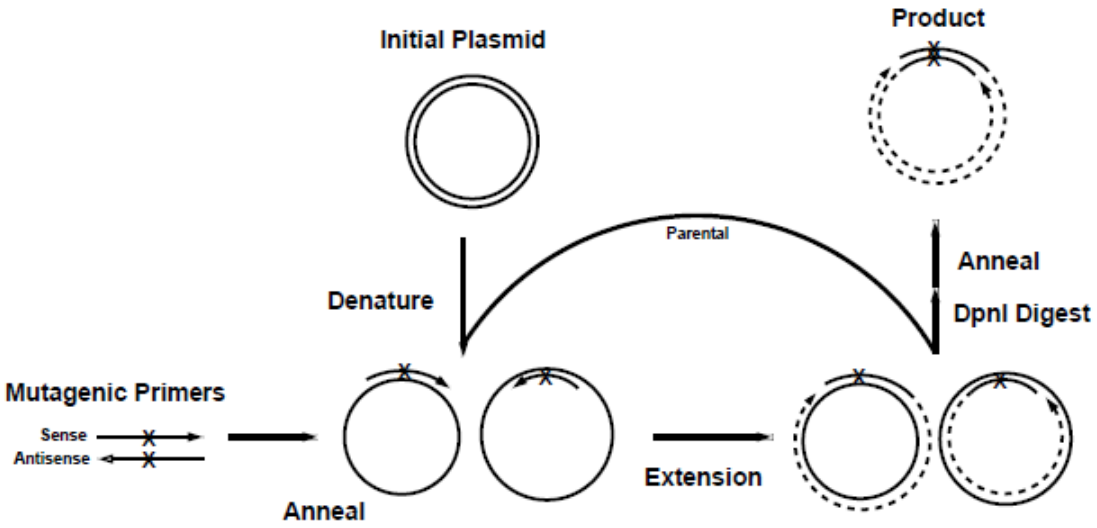
Site Directed Mutagenesis

This is the protocol for site directed mutagenesis based on the Stratagene protocole.



Materials:

- Pfu turbo
- 10X Pfu turbo buffer
- dNTPs (10mM)
- Forward and reverse primers (0.1ug/µL, see methods section for design tips)
- dH2O
- Dpn1
- Competent cells

PCR : See PCR protocol on the Wiki

Following PCR:

- Add 1uL of Dpn1 to PCR reaction.
- Incubate at 37°C for 1-2 hours to digest parental DNA.
- Run 5µL of the digested reaction on a gel and compare to the undigested parental plasmid (there should be some difference in band pattern).
- Transform into competent cells.

Following Transformation:

- Pick a colony, miniprep, and sequence to check for your mutation and any PCR introduced errors.

Trouble Shooting: If no product is seen, try repeating the protocol with 5% DMSO in the reaction mix. DMSO disrupts base pairing, facilitating strand separation in GC rich regions of DNA and reducing the propensity of the DNA to form secondary structure. The end effect, is a little DMSO will often get you past issues with poor primer design and/or difficult templates.